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Growth, water relations, and survival of drought-exposed seedlings from six maternal families of honey mesquite (*Prosopis glandulosa*): responses to CO_2 enrichment

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Summary Low water availability is a leading contributor to mortality of woody seedlings on grasslands, including those of the invasive shrub *Prosopis*. Increasing atmospheric CO_2 concentration could favor some genotypes of this species over others if there exists intraspecific variation in the responsiveness of survivorship to CO_2. To investigate such variation, we studied effects of CO_2 enrichment on seedling survival in response to uniform rates of soil water depletion in six maternal families of honey mesquite (*P. glandulosa* Torr. var. *glandulosa*). Three families each from the arid and mesic extremes of the species' distribution in the southwestern United States were studied in environmentally controlled glasshouses. Relative water content at turgor loss and osmotic potential were not affected by CO_2 treatment. Increased atmospheric CO_2 concentration, however, increased growth, leaf production and area, and midday xylem pressure potential, and apparently reduced transpiration per unit leaf area of seedlings as soil dried. Consequently, CO_2 enrichment about doubled the fraction of seedlings that survived soil water depletion. Maternal families of honey mesquite differed in percentage survival of drought and in several other characteristics, but differences were of similar or of smaller magnitude compared with differences between CO_2 treatments. There was no evidence for genetic variation in the responsiveness of survivorship to CO_2. By increasing seedling survival of drought, increasing atmospheric CO_2 concentration could increase the abundance of honey mesquite where establishment is limited by water availability. Genetic types with superior ability to survive drought today, however, apparently will maintain that advantage in the future.

Keywords: heritable variation, osmotic potential, water deficit, xylem pressure potential.

Introduction

The evolutionary consequences of CO_2 enrichment for plants has recently become the focus of much research, motivated in part by the prospect that increasing atmospheric CO_2 concentration might select for genotypes with high growth potentials. Intraspecific variation in the responsiveness of growth or reproduction to CO_2 enrichment has been identified in several non-cultivated plants (Wulff and Alexander 1985, Conroy et al. 1990, Curtis et al. 1994, Wayne and Bazzaz 1995, Zhang and Lechowicz 1995). If these differences result in differences in survivorship or fecundity, CO_2-responsive types could eventually dominate, resulting in altered mean population-level responses to CO_2 enrichment.

Studies of intraspecific variation at different atmospheric CO_2 concentrations have focused on genetic types that differ in growth rate or in sensitivity to nutrients or competition. However, water availability, rather than nutrients or competition, largely controls the distribution and abundance of plants at regional and higher scales (Stephenson 1990).

Morphological and physiological traits that allow plants to dominate when water is available become a liability when water is limiting. Consequently, traits that influence plant acquisition and use of water and tolerance of dehydration usually differ among species (Smith and Huston 1989) and genotypes within species from across gradients in water availability (Parker and Pallardy 1991, Zhang et al. 1997). Recent studies indicate that species that differ in sensitivity to drought and in associated morphological and physiological traits may not be equally advantaged by CO_2 enrichment when water is limiting (e.g., Picon et al. 1996). Such results imply that genotypes of a given species could also respond differently to CO_2 enrichment.

If rising CO_2 concentration is to change the abundances and distributions of genetic types, it must influence the establishment of new individuals. The most direct way in which CO_2 enrichment could influence seedling establishment is by altering seedling survivorship. Drought that is severe enough to limit survivorship, however, is thought to minimize or even eliminate the benefits of CO_2 enrichment to plants (Sage 1996).

We investigated effects of CO_2 enrichment on seedling survival in response to uniform rates of soil water depletion in six maternal families of the shrub honey mesquite (*Prosopis glandulosa* Torr. var. *glandulosa*), three families each from the arid and mesic extremes of the species' distribution. Honey mes-

quite is an aggressive invader of grasslands in the southwestern United States and northern Mexico and has increased greatly in abundance during the last two centuries (Johnston 1963, Archer 1989). The shrub is now the dominant woody plant on about 45 million ha of grazing lands that vary in annual rainfall from less than 200 to about 1000 mm (Johnson and Mayeux 1990). Three questions were of particular interest in this study. (1) Will CO_2 enrichment consistently increase seedling survivorship of drought? (2) Does honey mesquite exhibit intraspecific variation in ability to survive soil water deficit? (3) Will any such variation be affected by CO_2 enrichment? By examining genetic variation in the response of survivorship to CO_2, we attempted to address the influence of CO_2 concentration on selection in this species. Traits that could contribute to the postponement or tolerance of plant dehydration were measured to provide insight into mechanisms controlling variation.

Carbon dioxide enrichment has been shown to improve water relations of individually grown plants by reducing transpiration rates and the rate of soil water depletion (Rogers et al. 1984, Tolley and Strain 1985, Wray and Strain 1986). It is doubtful, however, whether seedlings on established grassland would benefit much by slowing water use, because "saved" water would probably be used by larger neighboring plants. We sought, therefore, to expose seedlings to similar rates of soil water depletion across CO_2 treatments.

Materials and methods

Experimental design and measurements

Seeds were collected during the summer of 1995 from three *P. glandulosa* plants near Temple and Waco in central Texas, USA (designated CT1, CT2, CT3; mean precipitation = 890 mm) and from three plants at the Jornada Experimental Range, north of Las Cruces in southern New Mexico, USA (designated J1, J2, and J3; mean precipitation = 200 mm). The shrub is obligately outcrossing, so seeds from a given plant are half-sibs (Simpson 1977). Collected seeds were used in two experiments in environmentally controlled glasshouses. Each experiment was designed as a two-way factorial with CO_2 concentration (ambient and elevated) and maternal family as factors. Families CT1 and J1 were studied during Experiment 1. The remaining four families were studied during Experiment 2.

During each experiment, honey mesquite was grown in a sandy loam soil in 0.05 m diameter and 0.6 m deep pots. This pot size facilitated the control and monitoring of soil water content, while permitting largely unrestricted development of honey mesquite taproots and realistic rates of soil water depletion. Pots were made from polyvinyl chloride pipe that was cut in half longitudinally to facilitate recovery of intact root systems. The two lengths of each pot were taped together before planting and secured at the base with a perforated cap. Each pot was weighed when empty and after it had been filled with air-dried soil. Three samples of the soil used to fill the pots were oven-dried at 100 °C for 72 h and weighed. The mass of soil added to each pot was calculated based on the mean ratio of oven-dried mass to air-dried mass of the three soil samples. Two days before planting, soil in each pot was wetted to drip by adding 100 ml of Hoagland's nutrient solution (Hoagland and Arnon 1950) and approximately 200 ml of water. Pots were weighed after drainage stopped. No additional water was added. Following seedling emergence, pots were weighed every 2 to 4 days to determine rates of soil water depletion. Soil relative water content (RWC) per pot was calculated by dividing the amount of water in the soil on each date (mass of soil plus water on each date minus mass of oven-dried soil) by the amount of water retained in soil after drainage ceased (mass of fully wet soil minus mass of oven-dried soil).

Two scarified seeds of a given family were planted in each pot. Seeds from families CT1 and J1 were each planted in 140 pots on April 15, 1996 (Experiment 1). Seeds of the remaining four families (CT2, CT3, J2, J3) were each planted in 80 pots on July 25, 1996 (Experiment 2). At planting, pots assigned to each family were randomly divided between glasshouse bays maintained at nominal CO_2 concentrations of 370 and 700 μmol mol^{-1}. To minimize any effect of bay on plant performance, plants and the appropriate CO_2 treatment were switched every two weeks between bays.

At emergence (Day 0), one seedling per pot was randomly designated as the target or experimental plant and the remaining seedling in each pot was removed. Two imbibed seeds of an additional (seventh) maternal family of honey mesquite were planted in every pot on Day 2 of each experiment. Leaf areas of these additional plants were manipulated by clipping during each experiment to equalize rates of soil water depletion across families and CO_2 treatments.

On Days 10, 20, 30 and 40 of Experiment 1 and Day 30 of Experiment 2, xylem pressure potential (Ψ_x) was measured near midday (1200–1400 h CST) with a pressure chamber (Model 3005, Soil Moisture Equipment, Golita, CA) on excised shoots of five randomly selected target plants from each maternal family and CO_2 treatment. Stems of some of the plants collected on Days 30 and 40 of Experiment 1 (n = 2–3 plants per family and CO_2 treatment) and Day 30 of Experiment 2 (n = 2 plants per family and CO_2 concentration) were recut under water, enclosed in plastic bags with the stem base immersed in water, and rehydrated overnight at 5 °C. The following day, xylem pressure potentials and fresh weights were measured as shoots dried in the laboratory to determine pressure–volume relationships. Values of osmotic potential at full hydration (π_{100}) and at the turgor loss point (π_0) and tissue relative water content at turgor loss (R_0) were derived from these data by standard methods (Schulte and Hinckley 1985, Koide et al. 1989). Briefly, π_{100} was derived from the linear regression of $1/\Psi_x$ on relative water content of the plant. Turgor loss point was estimated as described by Schulte and Hinckley (1985). The method uses deviation of measured values from the regression of $1/\Psi_x$ on relative water content and from points fitted by the regression in ratios to estimate the turgor loss point. Calculated parameters provide an indication of plant capacity for turgor maintenance as Ψ_x declines. Capacity for turgor maintenance is greater in plants with low π_{100} and highly elastic tissues, the latter characteristic may be manifested as low R_0. Immediately after determining Ψ_x, leaf

area of the remaining plants was measured with a photoelectric meter (LI-3000A, Li-Cor, Inc., Lincoln, NE).

At all harvests, roots were washed from soil and separated into lateral roots and taproots. All plant material was weighed after oven drying at 60 °C for 72 h. Leaves that were shed as soil water declined often fell outside the pots and could not be recovered, so leaf and plant biomass at each harvest included only the leaves that plants retained. Leaf area of the subset of plants on which pressure–volume relationships were measured was estimated by multiplying measured leaf mass by the mean specific leaf area of remaining plants harvested from the appropriate family and CO_2 treatment on the same date.

For each target plant, the total number of true leaves (excluding cotyledons) produced and the number of leaves that had senesced or been shed were recorded beginning on Days 28 and 38 of Experiments 1 and 2, respectively. A doubly pinnate leaf was considered to have been shed when > 50% of leaflets had been lost. A leaf was considered senescent if it remained attached to the plant, but was entirely brown.

Soil drying in the remaining pots was terminated by rewatering soil to drip on Day 66 of Experiment 1 and Day 82 of Experiment 2. The number and area of green leaves present were recorded for plants that obviously were alive at rewatering. Survival of the remaining plants was assessed 2 weeks later. Stems and roots of all plants were then harvested. Stem length above the cotyledonary node was measured, and roots were separated into lateral roots and taproots. All plant material was weighed after oven drying at 60 °C for 72 h. We excluded pots in which seedlings did not emerge or in which rates of soil water depletion differed greatly from those of the majority of pots.

The CO_2 control and environmental conditions

The CO_2 concentration and dewpoint temperature of air in each glasshouse bay were measured at 4-min intervals with a Li-Cor Model LI-6262 infrared gas analyzer. The CO_2 readings were corrected for atmospheric pressure measured with a Druck model DPI 260 pressure indicator (Druck, Inc., New Fairfield, CT). The infrared analyzer was calibrated daily against four CO_2 gas standards and monthly against a Li-Cor LI-610 dewpoint generator. Air temperature was measured in the center of each bay and outside the glasshouse with fine-wire (25-mm diameter) thermocouples. Photosynthetic photon flux density (PPFD) was measured on the glasshouse roof with a Li-Cor LI-190SB silicon photodiode and above the plants in each bay with Li-Cor LI-191SA silicon detectors along a 1 m-long sensing surface.

Pure CO_2 gas was injected into the appropriate bay as required to maintain the elevated CO_2 concentration. Air temperature within the air-conditioned bays was maintained near that of the outdoors by manually adjusting thermostatic controls. The CO_2 concentration of air in the ambient and elevated CO_2 treatments averaged 380 and 710 μmol mol^{-1} during Experiment 1 and 360 and 700 μmol mol^{-1} during Experiment 2. Standard deviations of CO_2 concentration were calculated daily. During the two experiments, the mean of these values ranged from 8.3 μmol mol^{-1} in the elevated CO_2 treatment to 28.1 μmol mol^{-1} in the ambient CO_2 treatment, where concentration was not directly controlled. Daytime mean temperatures increased from 27 to 29 °C during Experiment 1, but decreased from 30 to 24 °C during Experiment 2. Mean vapor pressure deficit of air during daylight was 1.3 kPa. The daily integral of PPFD inside the bays averaged 74% of that measured above the glasshouse during both experiments.

Statistics

Analysis of variance (ANOVA) with repeated measures (Potvin et al. 1990) was used to discern effects of CO_2 concentration and time since emergence (day) on leaf production and shedding during each experiment. Data collected at harvest on Days 10 through 40 of Experiment 1 were analyzed with a three-way ANOVA that included CO_2 treatment, family identity, harvest date, and appropriate interaction terms (Sokal and Rohlf 1981). Two-way ANOVA with CO_2 treatment, family, and a $CO_2 \times$ family interaction was used to analyze data from harvests on Day 30 of Experiment 2 and following each experiment. Significant differences among three or more means were assessed with Student-Newman-Keuls multiple range test. Bonferroni-adjusted significance levels were used in single degree of freedom comparisons within harvest date. Data were transformed before analysis when required to satisfy assumptions of ANOVA. Variable means are presented for individual treatments (family, CO_2) only when statistical interactions with other treatments were not significant ($P > 0.05$). Effects of CO_2 concentration on seedling survival were analyzed with likelihood ratio chi-square tests based on a logistic regression model. The four families in Experiment 2 were ranked by percentage survival at each CO_2 concentration. Spearmans rank correlation test was used to analyze stability of ranks between CO_2 treatments.

Results

Half-sibs from honey mesquite (*P. glandulosa*) trees growing near the mesic and arid extremes of the species' distribution were exposed to similar rates of soil water depletion at current ambient and elevated CO_2 concentration (Figure 1) by manipulating the leaf area of the two additional honey mesquite plants in each pot. In both experiments, soil RWC declined to about 0.25 during the first 30 days, then decreased slowly thereafter. Elevated CO_2 concentration apparently reduced transpiration per unit leaf area early in each experiment because it was necessary to retain the additional plants longer in the elevated CO_2 treatment than in the ambient CO_2 treatment to achieve similar rates of water depletion. During Experiment 1, for example, shoots of all additional plants per pot were removed from the ambient CO_2 treatment by Day 20, whereas shoots of additional plants were retained in most pots in the elevated CO_2 treatment until Day 35.

Experiment 1

Elevated CO_2 concentration increased root, shoot, and total biomass of families CT1 and J1 by slightly more than 20% over Days 10–40 following emergence (Table 1). Leaf area per

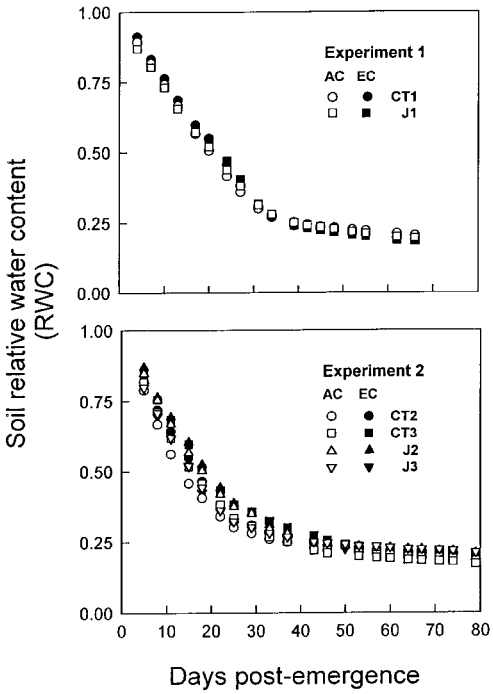

Figure 1. Mean soil relative water content (RWC) at two CO_2 concentrations (AC = ambient CO_2, EC = elevated CO_2) following emergence of *P. glandulosa* seedlings (Day 0) from six maternal families (CT1, CT2, CT3, J1, J2, J3). Watering was discontinued in each of the two experiments after wetting soil to drip on Day 0. Because plants were harvested during each experiment, the sample size of each mean declined from 70 at the first measurement to 48 to 50 on Day 40 of Experiment 1 (upper panel) and from 40 at first measurement to 17 to 35 on Day 30 of Experiment 2 (lower panel).

plant increased, but leaf area ratio (LAR; ratio of leaf area to total biomass) declined at high CO_2. Elevated CO_2 increased root biomass and both stem biomass and stem height of plants during the 66-day drying period, and more than doubled the leaf area of plants that survived the drought.

Family differences in growth were similar in magnitude to those between CO_2 treatments (Table 1). Seedlings from the tree in relatively mesic central Texas (CT1) were significantly larger and had greater mean leaf area and LAR over Days 10 to 40 of the drought period than seedlings from the tree near the arid extreme of the species' distribution (J1). The ratio of lateral root biomass to total root biomass was lower in CT1 seedlings than in J1 seedlings during the 66-day drying period.

Elevated CO_2 significantly increased midday Ψ_x (0.4 MPa less negative) across families over Days 10 through 40 (Table 1). Family differences in water relations did not become significant until Day 40 of the drought period ($P = 0.003$) when Ψ_x was 1.4 MPa lower in seedlings from the tree in central Texas ($\Psi_x = -5.32$ MPa) than in seedlings from more arid New Mexico ($\Psi_x = -3.91$ MPa). Neither osmotic potential at full hydration (π_{100}), potential at the turgor loss point (π_0), nor relative water content at turgor loss (R_0) differed between families, CO_2 concentrations (Table 2), or harvest dates (not shown).

Leaf production and loss differed significantly among families and CO_2 treatments (Figure 2). Seedlings of family CT1 produced more leaves during the experiment than seedlings of family J1. Elevated CO_2 generally increased leaf production in both families, although the effects depended on time of sampling. Elevated CO_2 increased the numbers of leaves that were lost or shed in family CT1, but did not affect leaf loss in the

Table 1. Growth, xylem potential, survival, and biomass distribution patterns of *P. glandulosa* seedlings grouped by CO_2 treatment (ambient, elevated concentration) or by maternal family (CT1, J1). Values shown are means (± SE). Seedlings were harvested during (Days 10 to 40; $n = 40$ per table entry) or following (Day 66; $n = 100$ and 98 per entry at ambient and elevated CO_2 and 100 and 98 per entry for families CT1 and J1, respectively) soil water depletion from fully hydrated conditions. Statistical significance of parameter differences between CO_2 treatments or families is indicated by the *P*-value (ns = not significant). When present, statistically significant interactions between treatments are indicated by: * = CO_2 × date interaction was significant ($P < 0.05$); ** = family × date interaction was significant ($P = 0.025$). Arithmetic means are shown, but most statistical analyses were performed after logarithmic transformation; $n = 49$ and 86 per table entry at ambient and elevated CO_2 and 59 and 76 per table entry for families CT1 and J1, respectively.

Parameter	CO_2 treatment		*P*-value	Family		*P*-value
	Ambient	Elevated		CT1	J1	
Days 10–40						
Root biomass (g)	0.100 (0.010)	0.123 (0.011)	0.0001*	0.120(0.011)	0.103(0.010)	0.005
Shoot biomass (g)	0.134 (0.009)	0.161 (0.011)	0.002*	0.160 (0.011)	0.134 (0.009)	0.002
Total biomass (g)	0.233 (0.019)	0.284 (0.021)	0.0003	0.280 (0.022)	0.237 (0.018)	0.016
Leaf area (cm^2)	10.98 (0.93)	11.90 (0.73)	0.04	13.57 (0.93)	9.26 (0.54)	0.0001
Leaf area/total biomass ($m^2\ g^{-1}$)	0.0052 (0.0003)	0.0046 (0.0002)	0.013	0.0054 (0.0003)	0.0044 (0.0003)	0.002
Xylem potential (MPa)	−2.33 (0.28)	−1.92 (0.24)	0.005	−2.39 (0.29)	−1.87 (0.22)	0.002**
Day 66						
Root biomass (g)	0.194 (0.006)	0.248 (0.006)	0.0001	0.233 (0.007)	0.207 (0.005)	0.001
Stem biomass (g)	0.093 (0.002)	0.142 (.003)	0.0001	0.113 (.004)	0.122 (.003)	0.03
Stem height (cm)	5.86 (0.12)	8.30 (0.18)	0.0001	7.99 (0.18)	6.04 (0.15)	0.0001
Lateral root/total root biomass	0.30 (0.01)	0.32 (0.01)	0.06	0.29 (0.01)	0.33 (0.01)	0.01
Leaf area/surviving plant[1] (cm^2)	2.47 (0.33)	6.50 (0.40)	0.0001	4.55 (0.46)	5.42 (0.46)	ns
Seedling survival (%)	47.6	87.8	0.0001	56.7	78.3	0.0006

Table 2. Tissue osmotic potential at full hydration (π_{100}; MPa) and at the turgor loss point (π_0; MPa) and relative water content at turgor loss (R_0) for seedlings of *P. glandulosa* harvested 30 and 40 days after emergence (Experiment 1; $n = 10$) or 30 days after emergence (Experiment 2; $n = 7$ per CO_2 treatment, 4 for families CT2, CT3, and J2, and 2 for family J3). Values shown are means (± SE). Parameters did not differ significantly between CO_2 concentrations or among families.

Treatment	π_{100}	π_0	R_0
Experiment 1			
CO_2 Concentration			
Ambient	−1.44 (0.17)	−2.10 (0.25)	0.81 (0.02)
Elevated	−1.55 (0.14)	−2.23 (0.18)	0.77 (0.03)
Family			
CT1	−1.64 (0.18)	−2.21 (0.24)	0.81 (0.03)
J1	−1.33 (0.07)	−2.13 (0.16)	0.77 (0.03)
Experiment 2			
CO_2 Concentration			
Ambient	−1.04 (0.16)	−1.59 (0.24)	0.78 (0.04)
Elevated	−1.40 (0.15)	−1.93 (0.19)	0.84 (0.01)
Family			
CT2	−1.10 (0.17)	−1.73 (0.15)	0.79 (0.07)
CT3	−1.61 (0.17)	−2.13 (0.24)	0.84 (0.01)
J2	−1.09 (0.24)	−1.71 (0.39)	0.78 (0.04)
J3	−0.96 (0.36)	−1.18 (0.44)	0.88 (0.01)

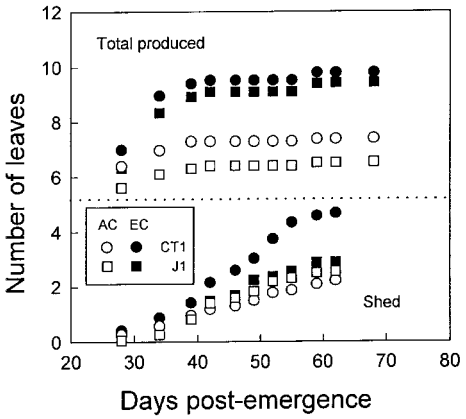

Figure 2. Total number of leaves produced and number of leaves lost or shed by seedlings (n = 48 to 50) of two maternal families of *P. glandulosa* (CT1, J1) as soil water content declined at each of two CO_2 concentrations (AC = ambient CO_2, EC = elevated CO_2).

smaller J1 seedlings. By the end of the drought period, the increase in leaf production in the elevated CO_2 treatment had been offset by an increase in leaf shedding among CT1 seedlings. In contrast, drought-exposed J1 seedlings retained more leaves in the elevated CO_2 treatment than in the ambient CO_2 treatment.

The fraction of seedlings that survived the 66-day drying period nearly doubled in response to CO_2 enrichment (Table 1). Percentage survival was higher among seedlings from the tree in New Mexico (J1) than among seedlings from the tree in more-mesic central Texas (CT1), but this difference was smaller than the difference between CO_2 treatments. There was no evidence of a significant $CO_2 \times$ family interaction for percentage survival.

Experiment 2

Elevated CO_2 concentration increased growth and improved water relations of honey mesquite seedlings during Experiment 2 (Table 3), but there were few differences in measured parameters among the four families studied and little evidence for differential effects of CO_2 enrichment on families. Leaf area and total biomass were increased more than 25% by CO_2 enrichment across families on Day 30 of the drying period. On Day 30, leaf area per plant was highest in family CT-2, but did not differ significantly among the remaining three families (data not shown). Biomass distribution patterns, measured as LAR and the ratio of lateral root biomass to total root biomass, did not differ between CO_2 concentrations (Table 3) or among families on Day 30 (data not shown). Xylem pressure potential was 1.2 MPa less negative at elevated CO_2 than at ambient CO_2 (Table 3), but did not differ significantly ($P = 0.54$) among families (means ranged from −2.2 to −3.0 MPa). None of the differences among families or between CO_2 treatments in π_{100}, π_0, or R_0 was statistically significant (Table 2), perhaps because of the relatively small sample sizes.

The stimulating effect of CO_2 enrichment on growth was evident in seedlings re-watered at the end of the 82-day

Table 3. Growth, xylem potential, survival, and biomass distribution patterns of four maternal families of *P. glandulosa* (CT2, CT3, J2, and J3) grown at ambient and elevated CO_2 concentration. Values shown are means (± SE). Seedlings were harvested during (Day 30; $n = 20$ per table entry) or following (Day 82; $n = 104$ and 131 per entry at ambient and elevated CO_2) soil water depletion from fully hydrated conditions. Statistical significance of differences between CO_2 treatments is indicated by the P-value (ns = not significant). An asterisk indicates that the $CO_2 \times$ family interaction was significant ($P = 0.003$). Arithmetic means are shown, but statistical analysis on some parameters was performed after transformation.

Parameter	CO_2 Treatment		P-value
	Ambient	Elevated	
Day 30			
Total biomass (g)	0.30 (0.02)	0.40 (0.03)	0.013
Leaf area (cm^2)	11.39 (1.00)	14.52 (1.37)	0.026
Lateral root/total root biomass	0.48 (0.03)	0.45 (0.05)	ns
Leaf area/total biomass ($m^2\ g^{-1}$)	0.0039 (0.0004)	0.0037 (0.0002)	ns
Xylem potential (MPa)	−3.18 (0.38)	−1.95 (0.14)	0.008
Day 82			
Root biomass (g)	0.167 (0.003)	0.229 (0.008)	< 0.001
Stem biomass (g)	0.059 (0.001)	0.086 (0.004)	< 0.001
Stem height (cm)	5.52 (0.11)	6.68 (0.22)	< 0.001
Lateral root/total root biomass	0.164 (0.008)	0.223 (0.007)	< 0.001*
Seedling survival (%)	11.4	24.4	0.012

drought period (Table 3). Elevated CO_2 concentration increased mean stem height by 21% and root biomass and stem biomass by 35 and 50%, respectively, across families. The fraction of root biomass present in lateral roots on Day 82 was higher at elevated CO_2 than at ambient CO_2 for each family, but the difference was significant only for family CT3 (data not shown). Families did not differ in root biomass, but differed in stem biomass and stem height. At the end of the drought period (Day 82), stems were taller in family CT3 than in other families (Table 4).

Elevated CO_2 concentration increased both the numbers of leaves produced and the numbers of leaves that died, but remained attached, or were shed (Figure 3). Consequently, CO_2 concentration did not affect the number of leaves retained per plant. Positive effects of elevated CO_2 on leaf production and turnover were significant across families, but differed with duration of drought. Families differed in leaf production, leaf loss to death and shedding, and leaf retention, although differences varied with time. Families differed less in the numbers of leaves that were lost than in leaf production. Consequently, leaf retention was lowest in the family (CT2) that produced the fewest leaves.

Mortality was proportionally higher in Experiment 2 than in Experiment 1, but the positive effect of CO_2 enrichment on survival was still evident (Table 3). Across families, CO_2 enrichment more than doubled percentage survival of seedlings following the drought period of Experiment 2. Mean percentage survival by family ranged from 5 to 24% at ambient CO_2 and from 18 to 38% at elevated CO_2, but family differences were not significant (P = 0.35) across CO_2 treatments (Figure 4). There was evidence from Spearmans rank correlation test that the order of families ranked by percentage survival differed between CO_2 treatments (P = 0.75 for the test of the hypothesis that there was no association between rank order of families at different CO_2 concentrations). The change in ranking, however, largely reflected a shift in family CT2 relative to the others.

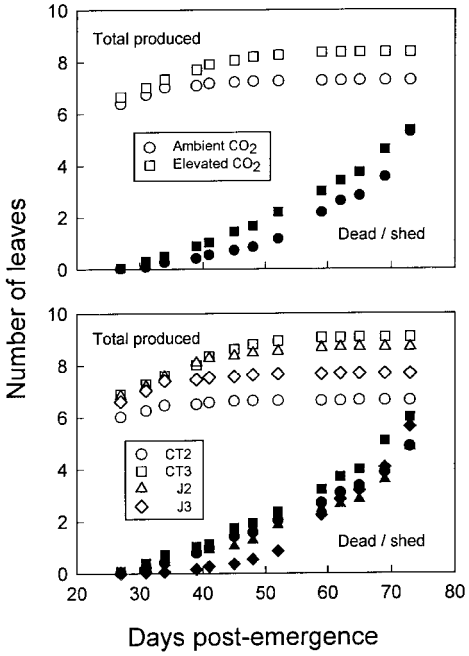

Figure 3. Total number of leaves produced and number of leaves that died, but remained attached, or were shed by *P. glandulosa* seedlings during soil water depletion, as affected by CO_2 treatment (upper panel, n = 104 and 131 at ambient and elevated CO_2) and maternal family (lower panel, n = 46 to 69).

Discussion

Plants usually suffer greatest mortality as seedlings. Because it is among the frequent causes of seedling mortality on grasslands, low water availability can be a formidable barrier to the establishment of *Prosopis* (Paulsen 1950, Ueckert et al. 1979) and other woody species (Harrington 1991, O'Connor 1995). Any factor that relieves drought stress and reduces drought-induced mortality could increase the abundance of water-limited genotypes on grasslands or extend their distribution into areas that formerly were too dry. Intraspecific variation in relief of drought stress could eventually lead to a change in the genetic composition of populations.

A high atmospheric CO_2 concentration is thought to ameliorate impacts of mild water limitation, but provide little benefit to severely droughted plants (Sage 1996). We found, however, that doubling CO_2 concentration approximately doubled the fraction of honey mesquite seedlings that survived soil water depletion. Although proportional survival was considerably lower after the longer drying period of Experiment 2 (82 days) compared with Experiment 1 (66 days), CO_2 enrichment still reduced mortality. This benefit of high CO_2 concentration apparently derived from changes in transpiration and growth that slowed the development of water stress in seedlings. Elevated CO_2 concentration may have increased plant access

Table 4. Root and stem biomass and stem height for *P. glandulosa* seedlings from four maternal families harvested following 82 days of soil water depletion from fully hydrated conditions (n = 46 to 69). Values shown are means (± SE) and are averaged across ambient and elevated CO_2 concentrations. Values within a row followed by the same letter did not differ significantly (Student-Newman-Keuls test).

Parameter	Family			
	CT2	CT3	J2	J3
Root biomasss (g)	0.199 (0.008) a	0.210 (0.011) a	0.222 (0.017) a	0.184 (0.006) a
Stem biomass (g)	0.062 (0.002) c	0.088 (0.005) a	0.079 (0.008) ab	0.072 (0.026) bc
Stem height (cm)	5.73 (0.13) b	7.40 (0.36) a	5.87 (0.37) b	5.82 (0.19) b

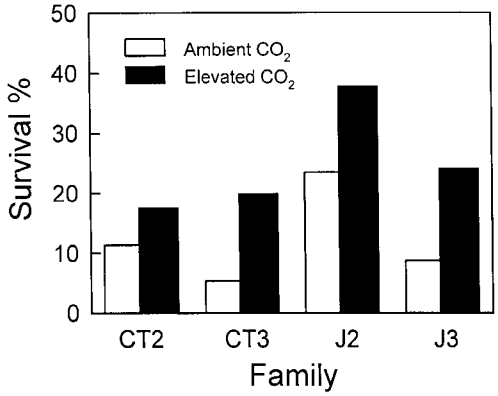

Figure 4. Percentage survival of *P. glandulosa* seedlings following 82 days without added water, as influenced by maternal family and CO_2 concentration (n = 17 to 35).

to water by increasing root growth and improved plant water balance by slightly increasing the fraction of root biomass invested in lateral roots. Similar changes have been noted in other studies (Tolley and Strain 1985, Miao et al. 1992, Polley et al. 1996). Probably as important as improved access to water were changes that reduced water loss. Transpiration per unit leaf area apparently declined at elevated CO_2, at least during the early stages of each experiment. Leaves were too small for gas exchange measurements, but the additional plants had to be retained longer in each pot at elevated CO_2 than at ambient CO_2 to deplete soil water at similar rates. In most families, CO_2 enrichment also increased leaf turnover when water limitation became severe, thereby, at least partially, offsetting the negative effects of greater total leaf production on plant water balance. It is possible that CO_2 enrichment also lowered the minimum Ψ_x that seedlings could tolerate. At least some plants survived midday Ψ_x more negative than −8.0 MPa, the minimum Ψ_x that we could measure. However, neither osmotic potentials nor relative water content at turgor loss was affected by CO_2 concentration in honey mesquite, a response contrary to that measured in some species (Sionit et al. 1981, Morse et al. 1993, but see Tschaplinski et al. 1993, 1995, Polley et al. 1996).

Maternal families of honey mesquite differed in several characteristics related to survivorship of drought, indicating the existence of genetic variation for these traits. Even among families selected from the extremes of the species' distribution, however, differences in traits and in survivorship were of similar (Experiment 1) or smaller magnitude (Experiment 2) than those between CO_2 treatments. In Experiment 2, for example, families did not differ significantly in midday Ψ_x, root biomass, or proportional survival of drought, all of which were improved by CO_2 enrichment. When families differed in drought survival, as in Experiment 1, differences in plant water status appeared to be related. Xylem pressure potentials were lower and mortality was higher among the faster-growing CT1 seedings than among the slower-growing J1 seedlings. A 45% greater leaf area apparently contributed to lower Ψ_x in CT1 seedlings than in J1 seedlings over Days 10 to 40. Not surprisingly, maintenance of less negative Ψ_x is often correlated with ability to survive drought (e.g., Gurevitch et al. 1986).

An expected negative relationship between precipitation at site of origin and percentage drought survival among families was not consistently supported. In each experiment, fractional survival at ambient CO_2 was highest in a family from the dry New Mexico site. However, at ambient CO_2 in Experiment 2, family differences in percentage survival were not significant, and survival of one family from New Mexico (J3) ranked below that of a family (CT2) from central Texas.

We found no evidence for significant $CO_2 \times$ family interactions for percentage survival, and little evidence for genetic variation in response to CO_2 for measured traits. Elevated CO_2 did change the order of families ranked by percentage survival in Experiment 2, but differences among families were small and not significant. Survival is directly related to fitnesss, so genetic types with a greater response to CO_2 should be favored in the future. Although the change in ranking of families by survival in Experiment 2 indicates the possibility of selection in extreme drought, the small and nonsignificant differences among families argue against its likelihood.

Clearly, the most dramatic result from this study was the highly positive effect of CO_2 enrichment on seedling survival of water depletion, a result that was evident across six maternal families from different environments. The extent to which this CO_2 effect will be expressed in the field, where soil water content might decline more slowly, remains to be assessed. We are aware of no published data on the water relations of field-grown honey mesquite seedlings during the first several months following emergence. It is evident from the literature, however, that honey mesquite is most vulnerable to drought during these initial months. Honey mesquite can rapidly extend its tap root into moist soil at depth, largely decoupling the water relations of older seedlings from water dynamics in surface soils (Brown and Archer 1990). Ueckert et al. (1979) reported that only 3 to 48% of emergent seedlings survived for 4 months during a dry year in Texas, USA, whereas survivorship after 2 years ranged from 0 to 17%. Our results suggest that rising CO_2 concentration could increase survival of drought-exposed honey mesquite seedlings, and thereby increase the abundance of honey mesquite in habitats where establishment is water-limited. Genetic types with superior ability to survive drought today, however, apparently will maintain that advantage in the future.

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